Personalized Medicine

Co-stimulation: considerations for CAR design

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NIH Safety Symposium

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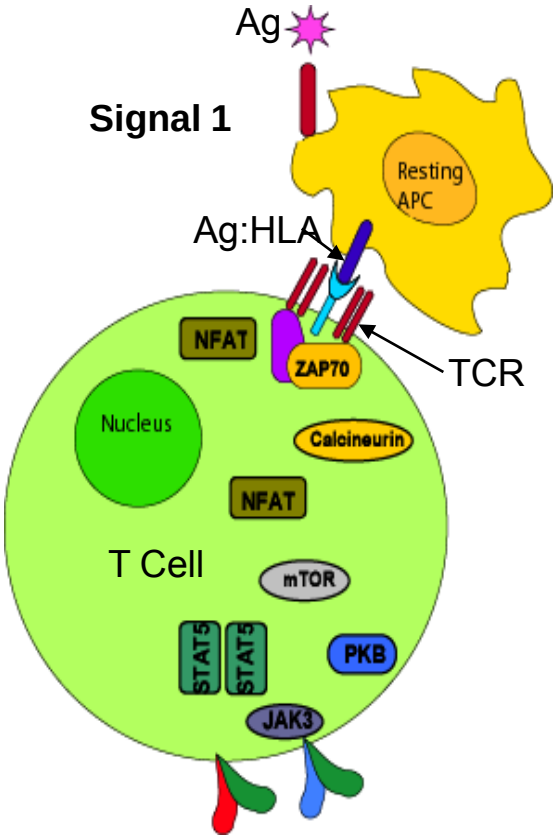
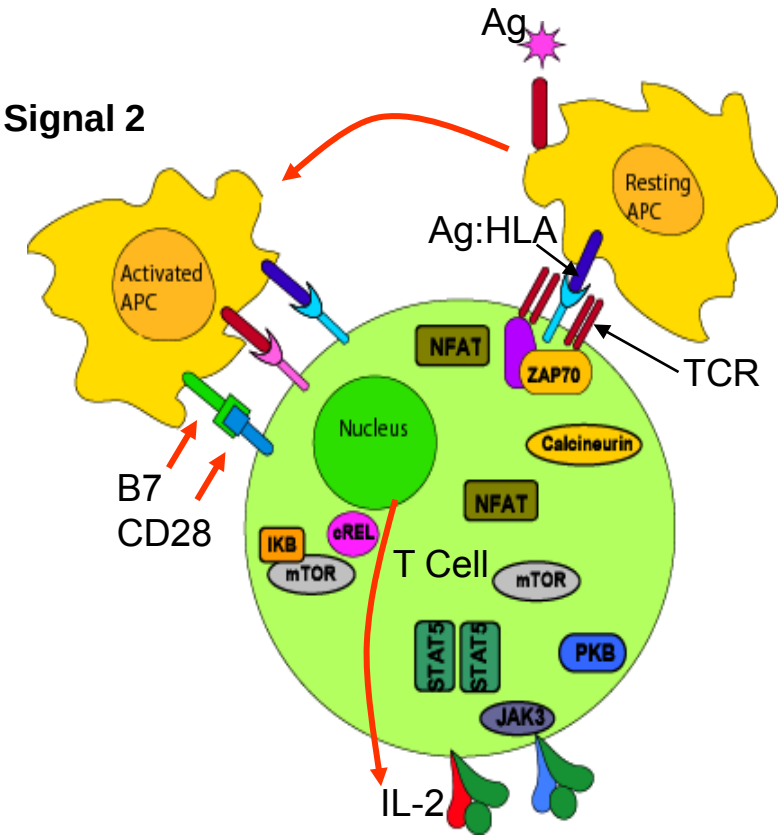
Penn Medicine

Abramson Cancer Center

T Cell Activation: antigenicity vs immunogenicity
The concept of costimulation



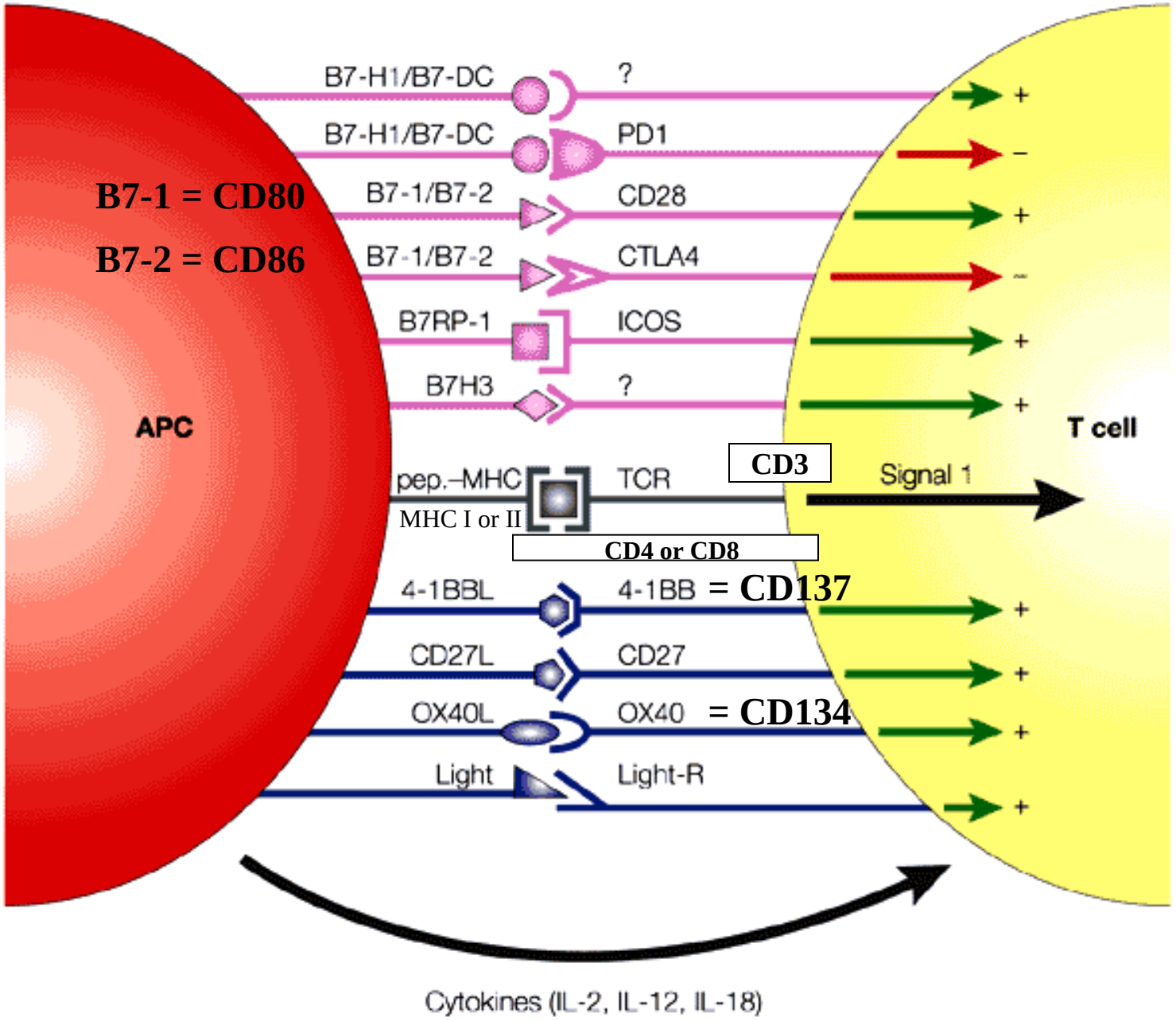


<u>Antigen:APC T cell interaction, no costimulation</u>

Result: T cell anergy, apoptosis or suppression (Treg)

<u>Antigen:APC T cell interaction with costimulation</u>

Result: T cell activation, clonal expansion effector functions

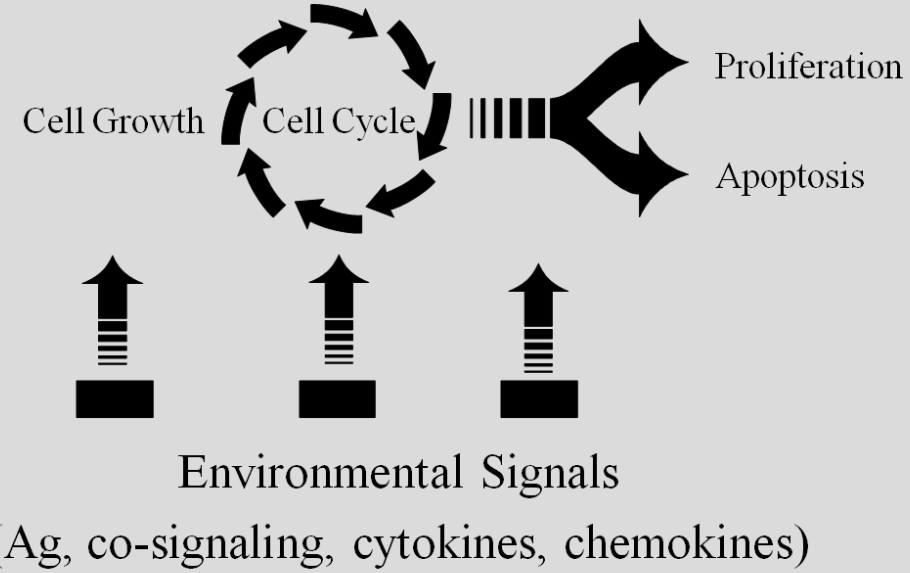


“Immunological synapse”

Nature Reviews | Immunology

Roles of CD28

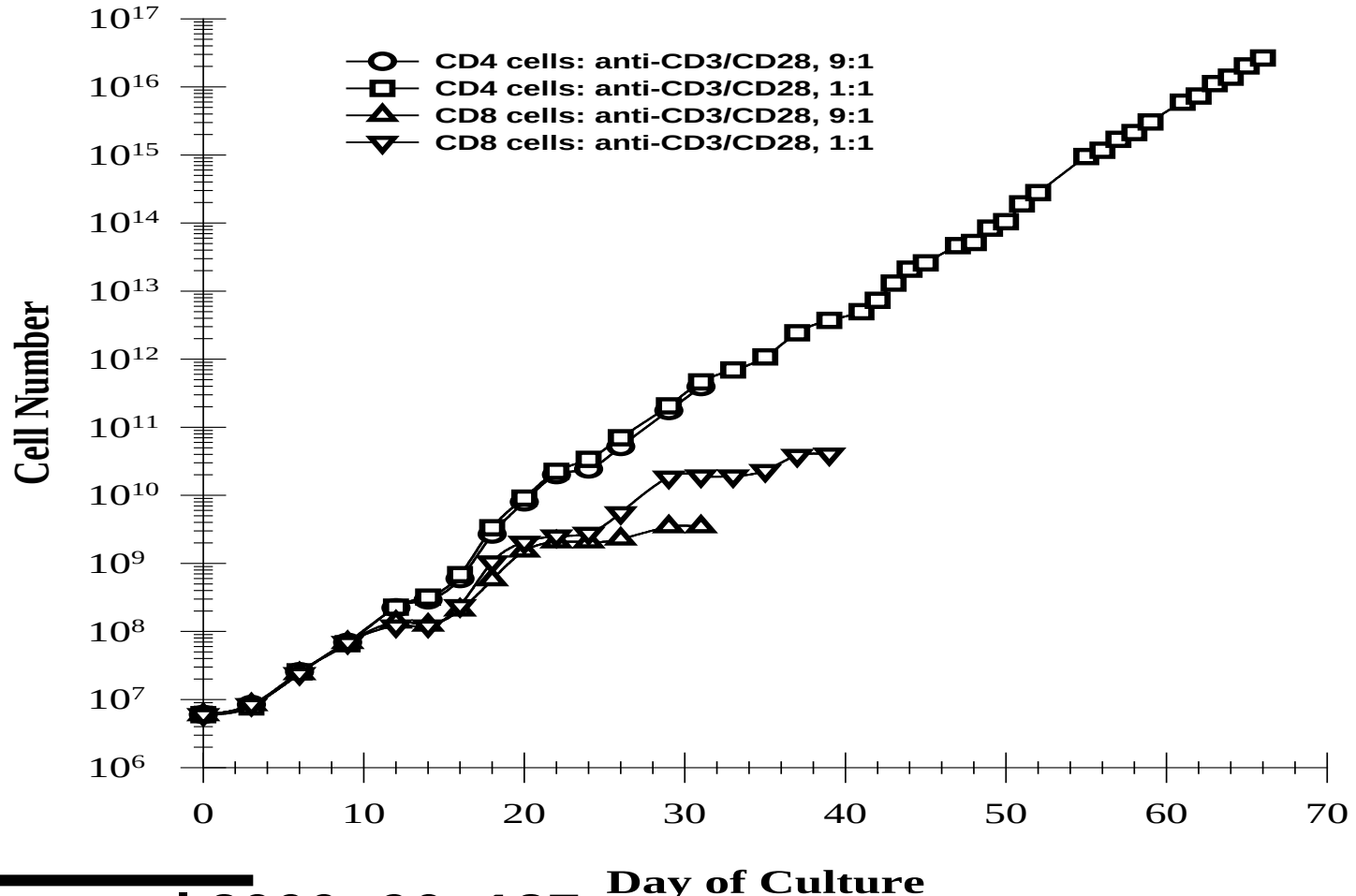
- Induction and maintenance of cytokine and chemokine secretion
- Cell survival: bcl-X induction and promotes clonal expansion
- Enhanced telomerase activity
- Required for T cells to increase their glycolytic rate (PI3K and Akt)
- Down regulation of beta chemokine receptor expression



Principles of costimulation co-signaling and co-inhibition

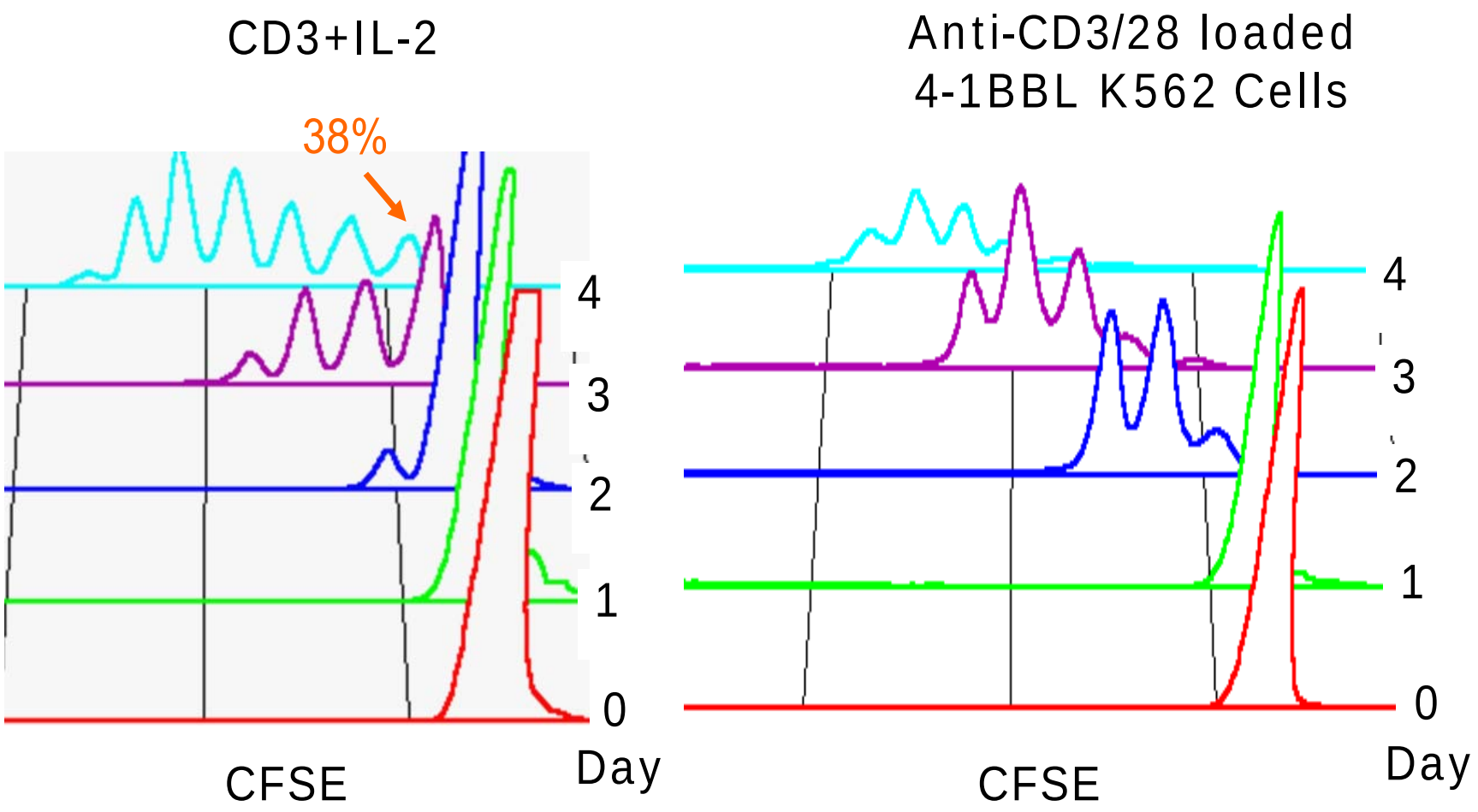
- Costimulatory signal transduction pathways downstream are conserved in mouse and man
- Expression of costimulatory molecules on lymphocyte subsets has substantial species specific variation (e.g. CD28 and ICOS)
- Lymphocyte subsets have distinct costimulatory requirements
  - CD28 is essential for CD4eff and Treg function
  - 4-1BB has a major role in CD8 Tcm function

Distinct Growth Requirements for Human CD4 and CD8 T Cells

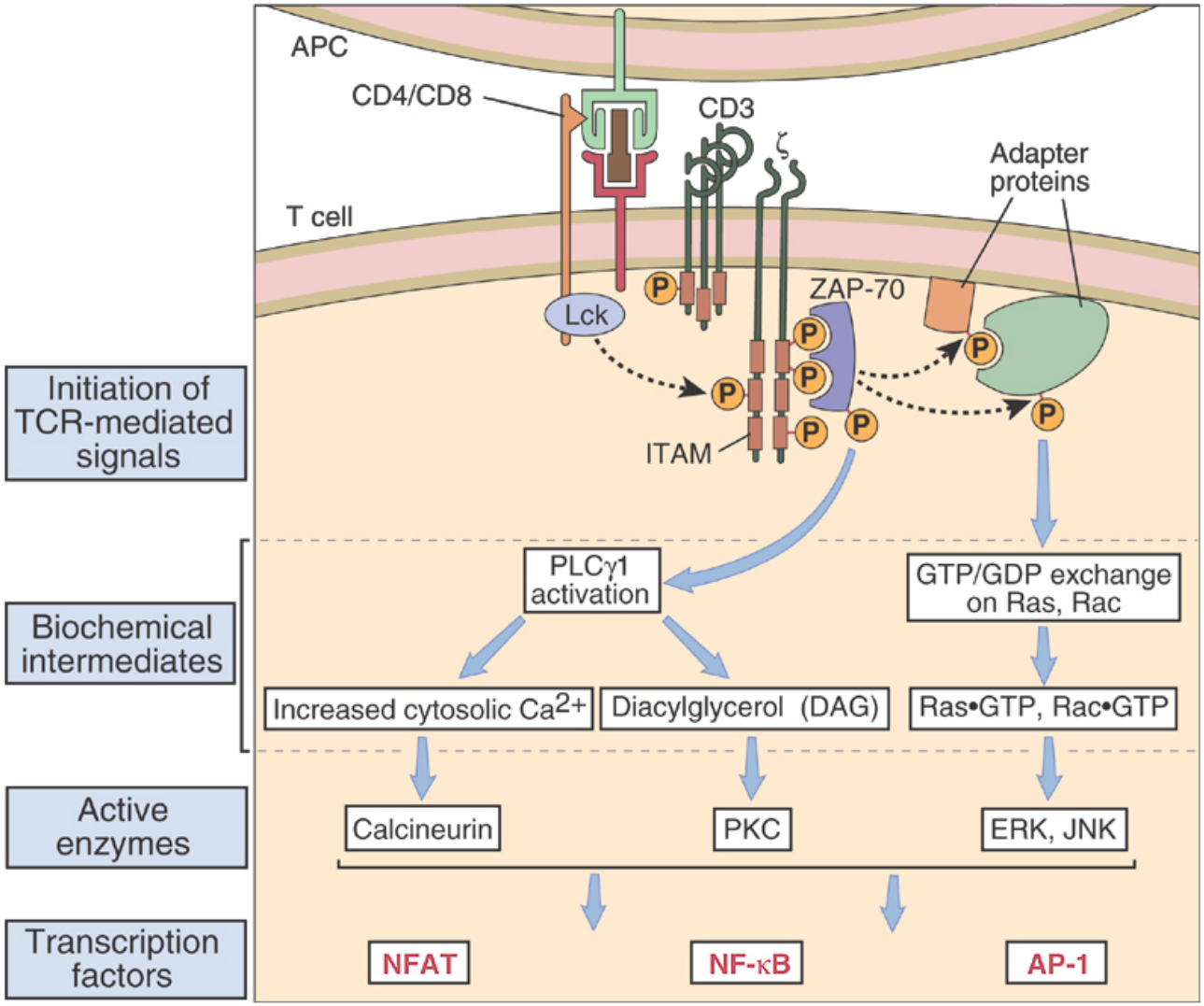


Clin Immunol 2000; 96: 187

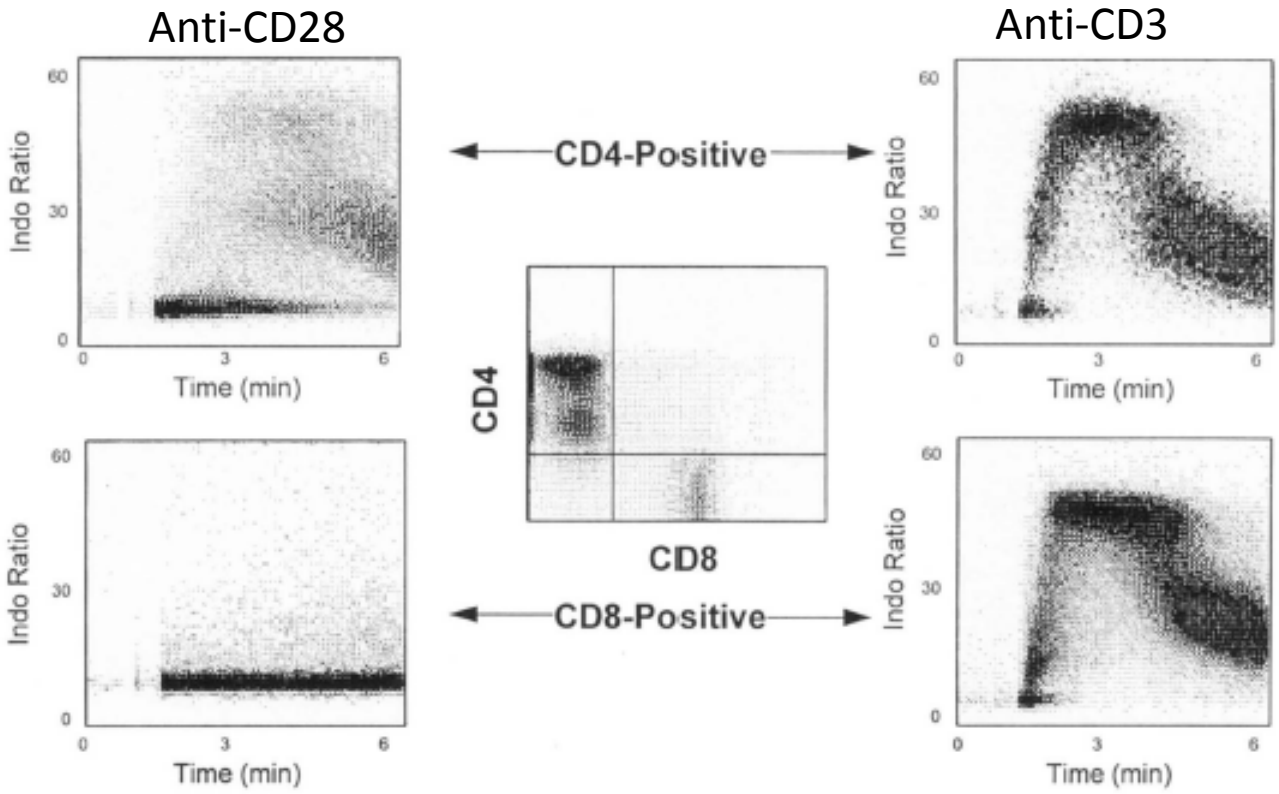
Efficient Induction of CD8 T Cell Proliferation by CD28:4-1BBL aAPC



T cell receptor-induced signal transduction pathways



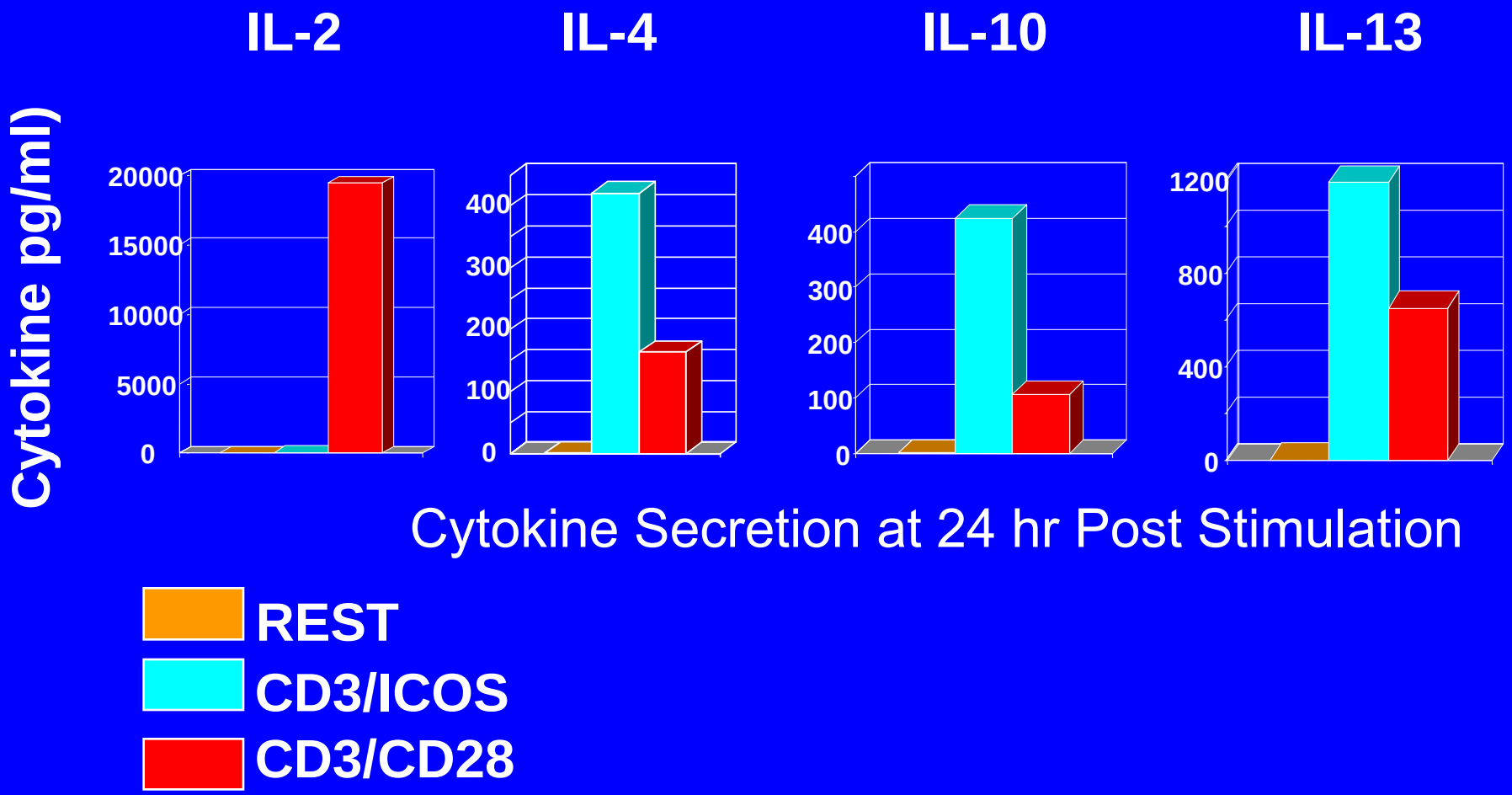
TCR and CD28: Distinct Signal Transduction in Splenic CD4 and CD8 T Cells



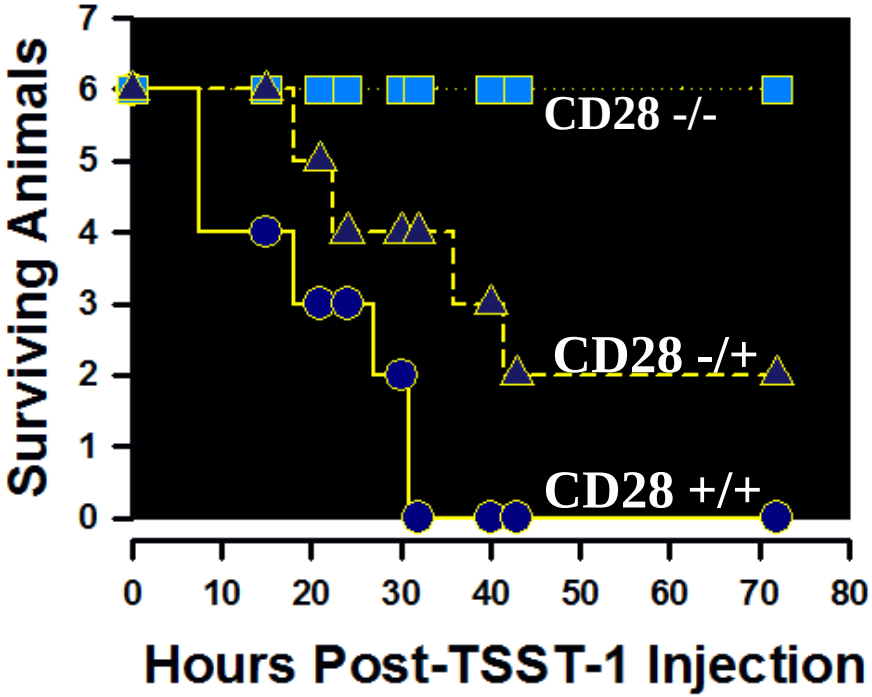
- **All mouse T cells express CD3 and CD28**
- **All mouse T cells flux calcium after CD3/TCR stimulation**
- **Only CD4 T cells flux calcium after CD28 stimulation**

Abe et al, J Immunol 1995

Distinct Effects of CD28 and ICOS on T Cell Effector Functions: Cytokine Secretion



Synchronous CD28 triggering can cause cytokine shock in mice and man



Saha. 1996.JEM 183:2675

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412

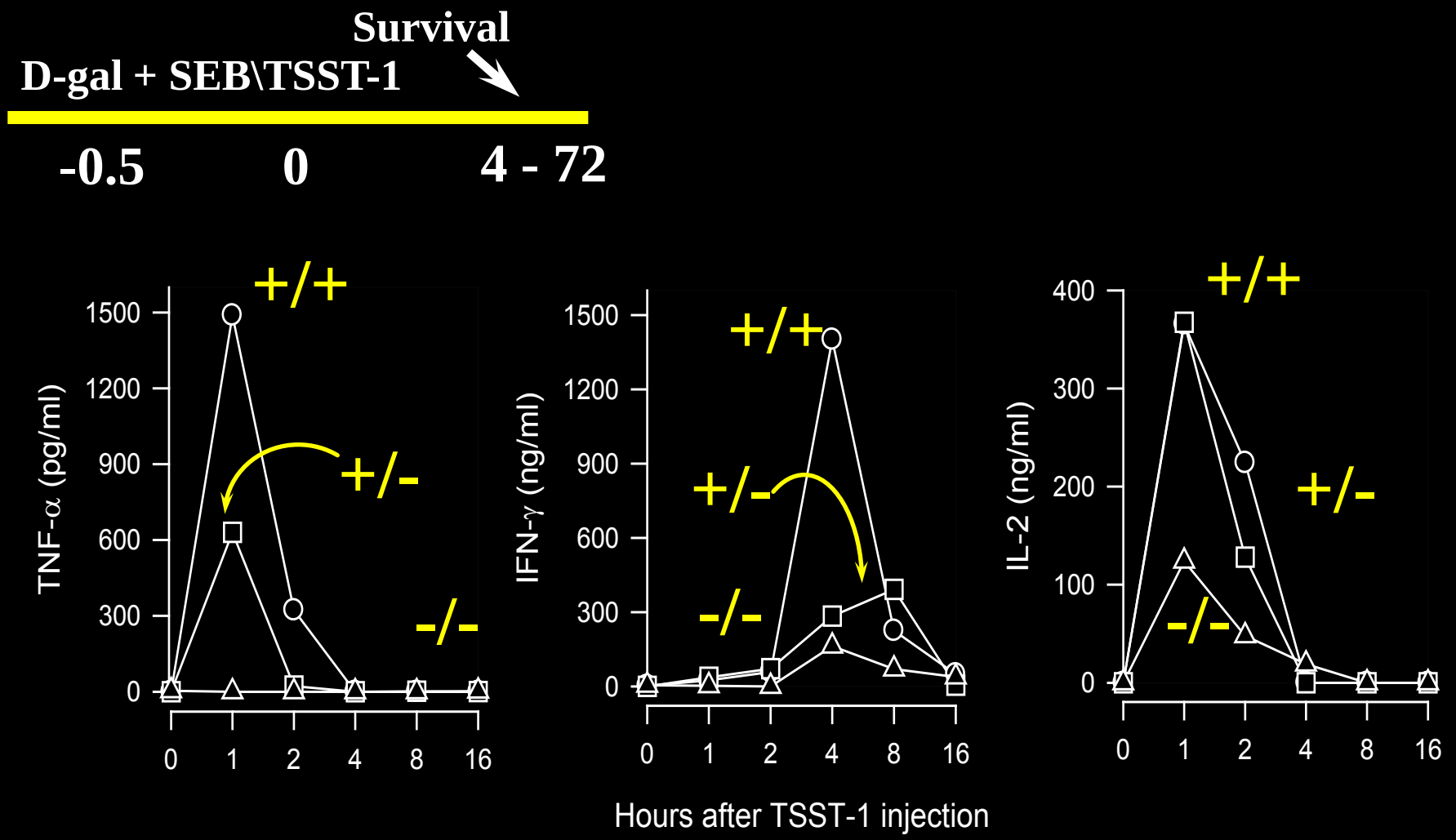
Ganesh Suntharalingam, F.R.C.A., Meghan R. Perry, M.R.C.P., Stephen Ward, F.R.C.A., Stephen J. Brett, M.D., Andrew Castello-Cortes, F.R.C.A., Michael D. Brunner, F.R.C.A., and Nicki Panoskaltsis, M.D., Ph.D.

SUMMARY

Six healthy young male volunteers at a contract research organization were enrolled in the first phase 1 clinical trial of TGN1412, a novel superagonist anti-CD28 monoclonal antibody that directly stimulates T cells. Within 90 minutes after receiving a single intravenous dose of the drug, all six volunteers had a systemic inflammatory response characterized by a rapid induction of proinflammatory cytokines and accompanied by headache, myalgias, nausea, diarrhea, erythema, vasodilatation, and hypo-

NEJM 2006.355: 1018

CD28 is required for mouse exotoxin shock: dose dependence for serum TNF-α, IFN-γ and IL-2



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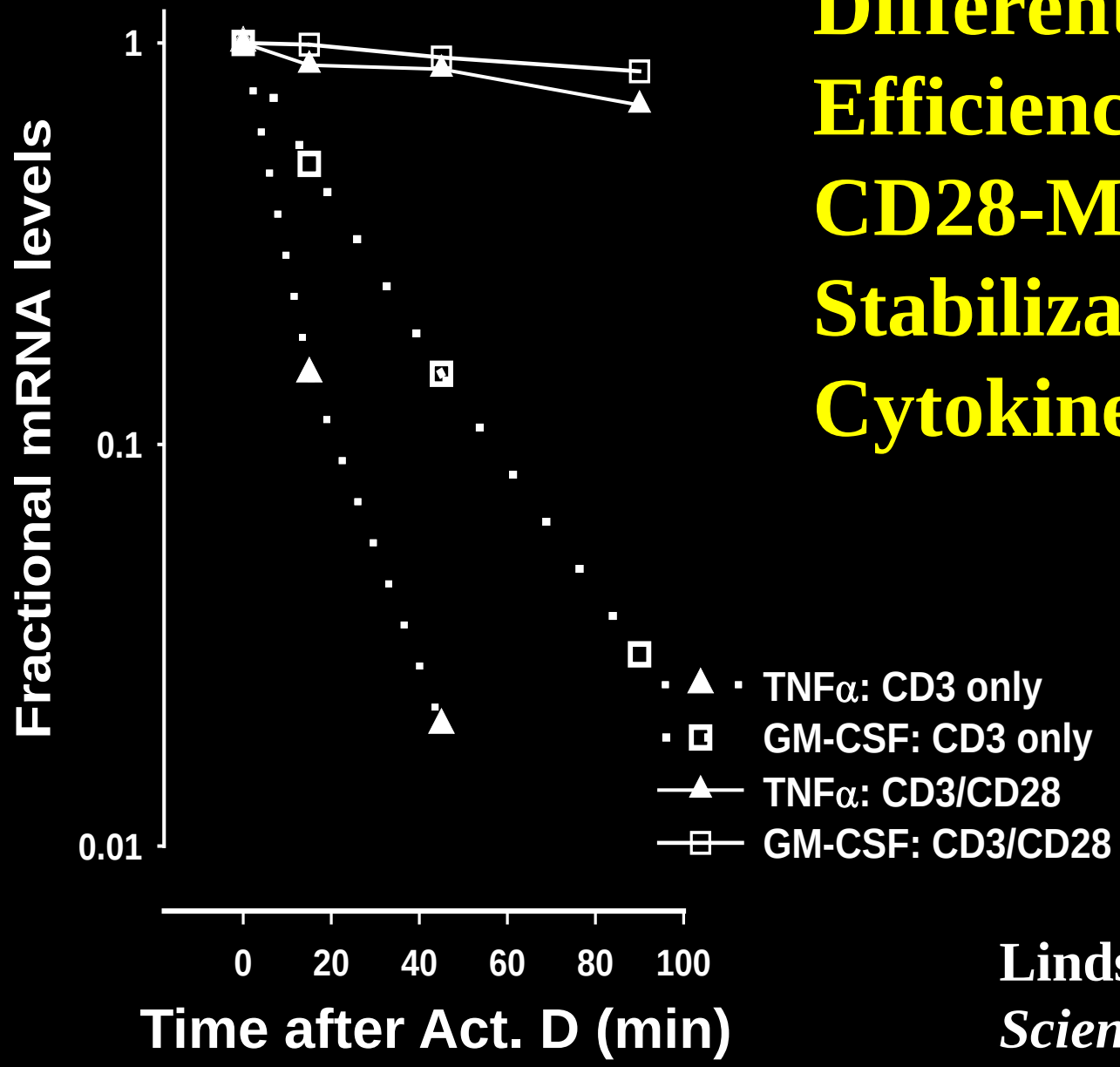
Differential Efficiency of CD28-Mediated Stabilization of Cytokine mRNA



Lindsten et al,
Science 1989; 244: 339

Triggering endogenous CD28 can cause severe cytokine release in mice and humans

- **Enterotoxin mediated shock in mice is CD28 dependent**
- **Humans develop cytokine release syndrome after infusion of agonistic CD28 mAbs**
- **Synchronous triggering predisposes**
- **Evidence for dose dependent effects**

Co-signaling domains in CARs

- **TCRζ or FcεR1**
  - **CARs with "first generation" TCRζ or FcεR1 epsilon trigger killing**
  - **In primary T cells, TCRζ signals in absence of costimulation lead to anergy**
- **CD28**
  - **Studies to date indicate that the signaling domain needs to be membrane proximal.**
  - **CD28 CARs have enhanced function and engraftment (pre-clinical and clinical data)**
  - **CD28 Y170F and P187/190A CARs have *attenuated* function (Darcy).**
  - **CD28 w mutated dileucine motif (LL => GG) have *increased* expression in mice (Geiger). Clinical studies pending.**

Co-signaling domains in CARs

- 4-1BB (CD137)
  - 4-1BB signaling in CARs promotes survival of CARs in tumor bearing mice. Clinical studies underway.
- Other costimulatory and kinases tested in CARs
  - OX40 (Finney, Pule)
  - ICOS (Finney)
  - NKG2D (Sentman)
  - Lck (Geiger)

Preclinical Evaluation – Gene Therapy and Immunotherapy Agents

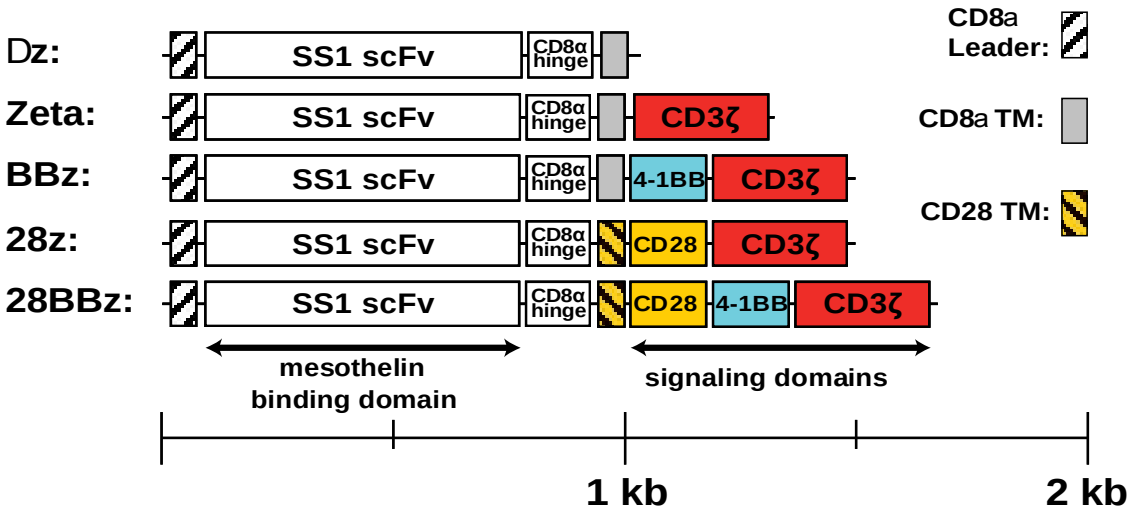
- BUT… the approach by which safety data are obtained will differ:

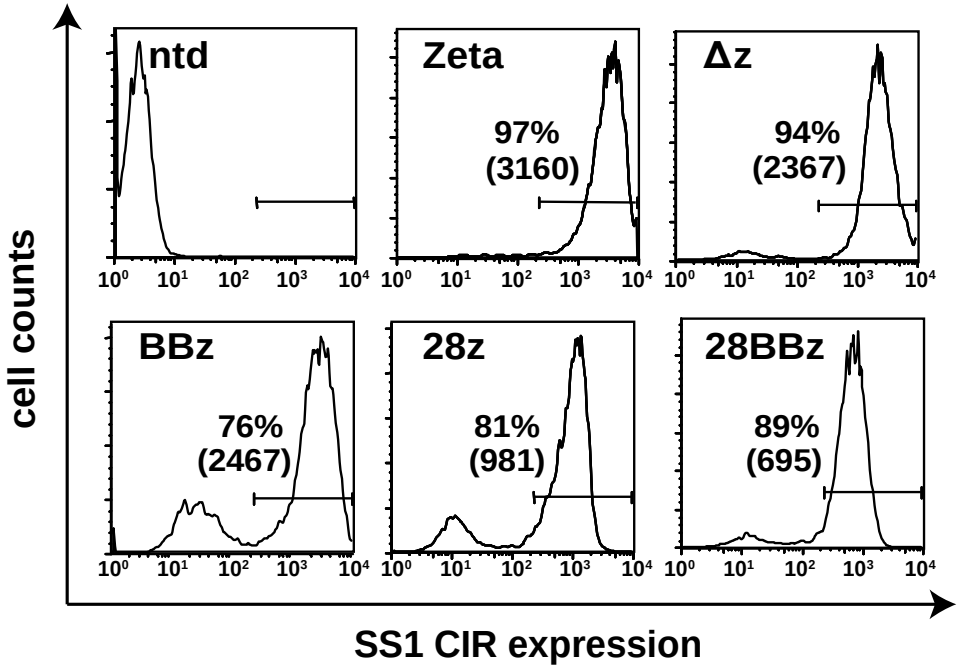
GT, IT (gene-based) …..	IT, Cell Products
➢ Biodistribution of vector/virus	➢ Immunogenicity to allogeneic cells
➢ Kinetics of gene expression	➢ Uncontrolled cell proliferation following *ex vivo* modifications

Ying Huang, Ph.D.

FDA/CBER/OCTGT/DCEPT/PTB

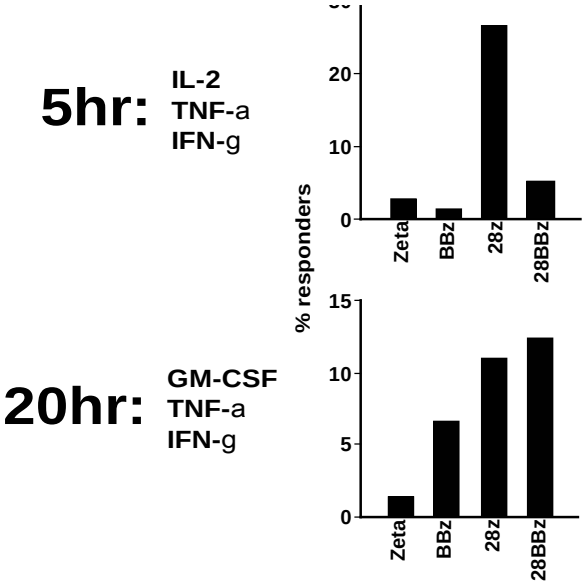
Lentiviral Redirected T Cells Targeting Mesothelin or CD19



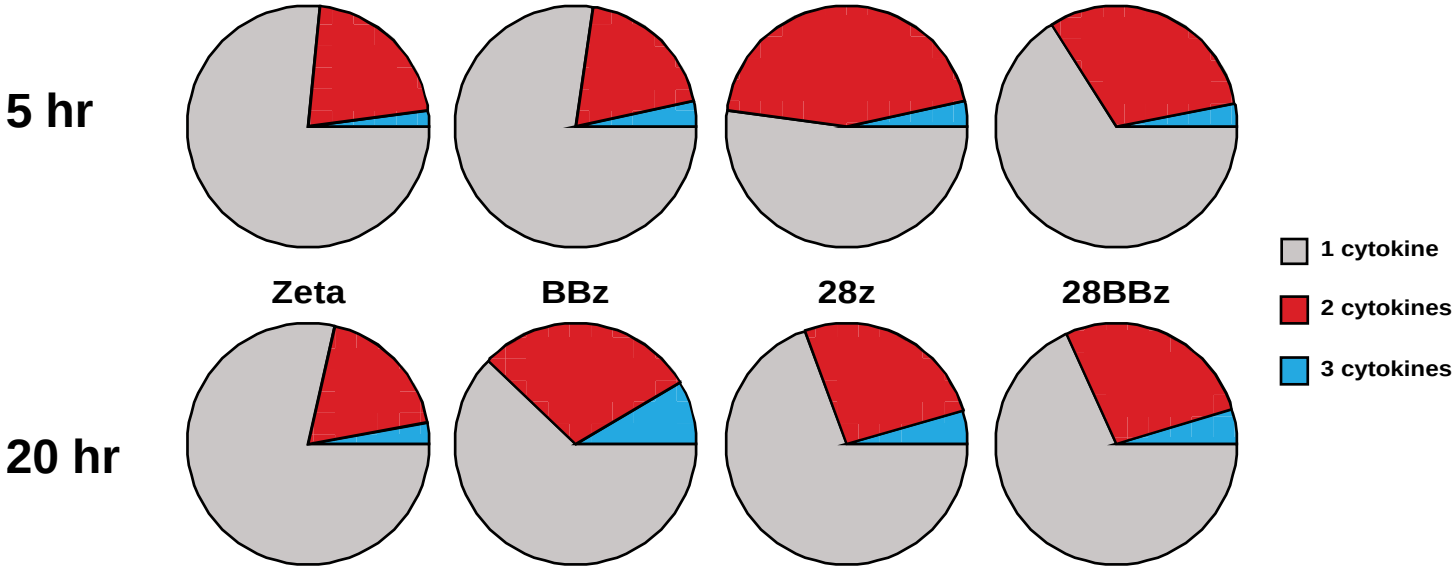


Carmine Carpenito
Michael Milone

Redirected T Cells with Costimulatory Domains are Multitasking "Polyfunctional" T Cells

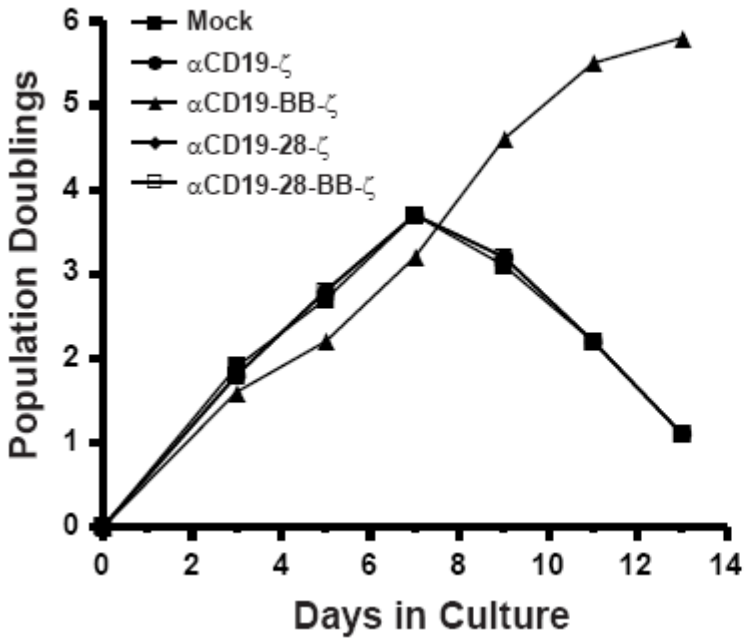


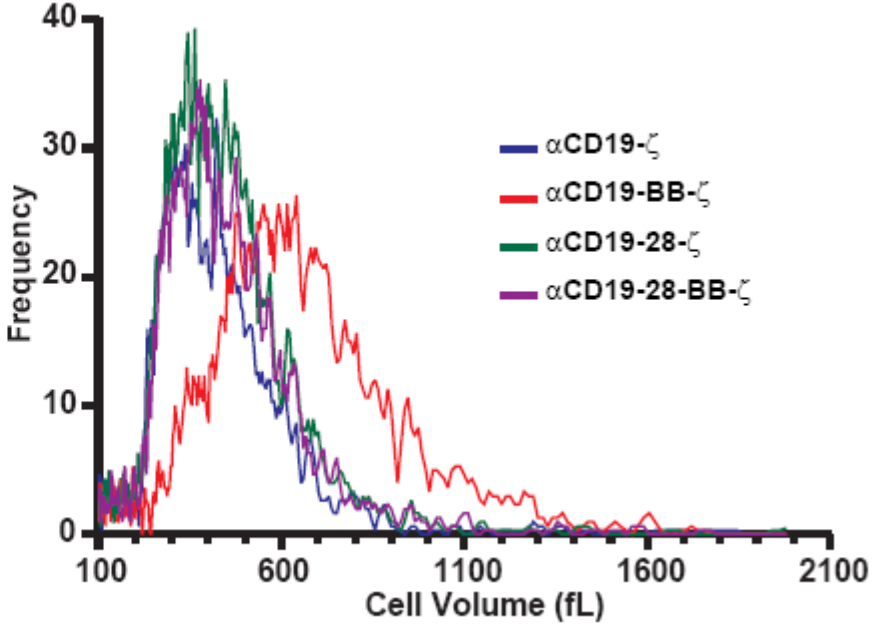
- Betts et al. 2006. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood 107:4781



4-1BB Signaling:αCD19 CAR-induced T cell proliferation in vitro antigen-dependent and antigen-independent effects

T cells	Stim CD3/CD28	LVV Transduction	IL-2 100u/ml
	0	1	5 - 14





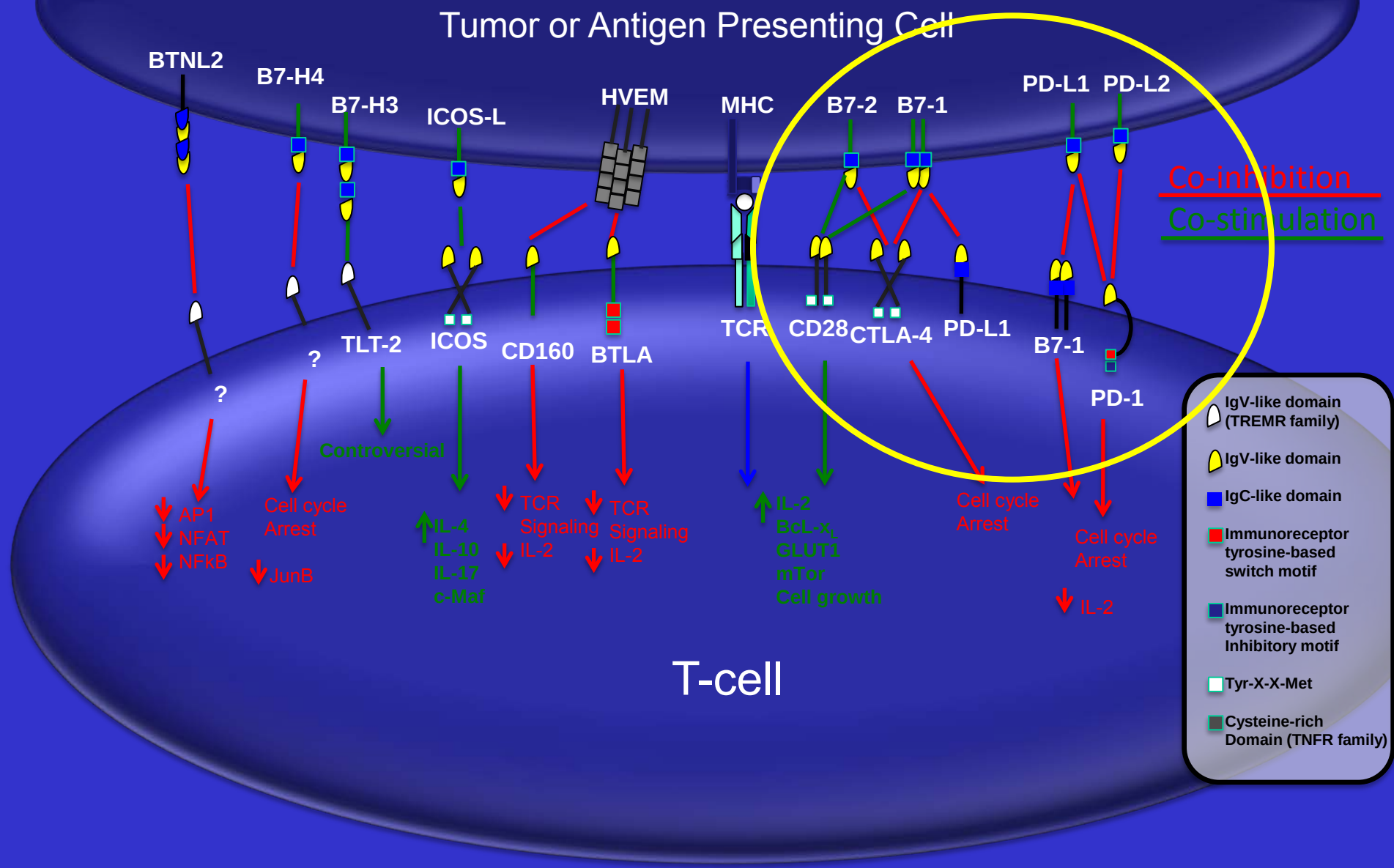
Combinatorial Signaling Domains

	Zeta only	28:z	41BB:z	28:41BB:z
kill	++	++	++	++
cytokine	+	+++	++	+++
Prolifera-tion	+	+++	+++	+++
In vivo survival	+	++	+++	+++

Reverse Signaling and Potential Complex Interactions with CAR Extracellular Domains

- Reverse or ‘back signaling’ has been reported for co-stimulatory molecules, particularly type II membrane proteins of TNFR superfamily
- CD28 reported to induce stimulatory signals in dendritic cells via CD80 and CD86 (Orabona, Nature Immunology 5: 1134-42, 2004)
- The physiologic significance of reverse signaling remains unknown:
  - Con: Generally poor sequence conservation of ligands, in comparison to highly conserved sequences of CD28 family
  - Pro: A casein kinase I motif present in the cytoplasmic domain of members of the TNF ligand family is implicated in 'reverse signaling‘ (Watts, EMBO Journal 18: 2119)

CD28 family members: potential complexities with reverse signaling and promiscuity



Strategies to regulate the potency of costimulatory CARs

1. **Inducible vs constitutive promoters**
   - **HIF1-α. (Makino et al. *J Immunol 171: 6534, 2003)***
2. **Conditional "suicide" switches**
3. **Non-integrating expression systems**
   - **mRNA transduction (Zhao et al. High-efficiency transfection of primary human and mouse T lymphocytes using RNA electroporation. *Molecular Therapy 13: 151, 2006)***
   - **Protein transduction and protein painting. (Chen et al. 2000. Hierarchical Costimulator Thresholds for Distinct Immune Responses: Application of a Novel Two-Step Fc Fusion Protein Transfer Method 1. *J Immunol 164: 705, 2000)***

Costimulatory CARs: Summary

1. **The potency of CAR T cell is a function of the cell and the CAR.**
2. **Incorporation of a variety of costimulatory signaling domains enhances anti-tumor functions of CARs.**
3. **The available studies suggest that the function of the signaling domains in CARs replicates endogenous costimulatory functions.**
4. **Synchronized activation of CARs should be avoided to limit the potential for cytokine release syndrome.**
5. **A variety of evidence suggests that co-stimulation may have non-redundant functions in various lymphocyte subsets. Given that the expression of co-stimulatory molecules on lymphocyte subsets may vary between mouse and humans, some clinical effects are difficult to predict from pre-clinical studies.**